Celkey® CD PK15 259

Version 1.1

Celkey® CD PK15 259 medium is a serum-free chemically defined medium. It does not contain animal-derived components, proteins, hydrolysates and other undefined components. which can support stable passage, high-density growth of PK-15 cells and related virus infection proliferation. It widely used in PCV (Porcine Circovirus), TGEV (Transmissible Gastroenteritis of Swine), PPV (Porcine Parvovirus) and PRV (Pseudorabies Virus) culture, proliferation, research and large-scale industrial production of related vaccines.

Product	Catalog No.	Form	Package Sizes
CD PK15 259	10701-259	Dry powder	2 L, 10 L, 100 L, Customized

Safety Warning

Read the Material Safety Data Sheets (MSDS) and follow handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Intended Use

For research and further manufacturing use only.

Component information

With 6.0 g/L glucose, 6.0 mM L-glutamine, sodium bicarbonate, phenol red, Pluronic F-68.

Storage and Stability

The dry powder medium should be stored at 2–8°C and protected from light. The product is stable for 24 mouths when unopened and stored properly. This product is very hygroscopic and should be kept in a dry environment away from moisture.

Prepare Liquid Medium from Dry Powder

1. Fill the mixing container with purified water (20–30°C) at 80%–90% of the final volume.
2. Slowly add 22.45 g/L of dry powder medium with gentle stirring. Mix for 30 minutes.
3. Add 0.49 mL/L of TE020 (99069-17017, shipped with CD PK15 259 medium). Mix for 10 minutes.
4. Adjust the pH to 6.8–7.0 using 1 M–5 M NaOH or HCl. The pH may rise 0.1 to 0.2 after sterile filtration.
5. Adjust the final volume with purified water. Mix for 10 minutes.
6. Filter the media using a membrane filter with 0.22 μm pore size immediately.

Culture Conditions

Medium: CD PK15 259
Cell line: PK-15
Inoculation density: 0.5-0.6 × 10^6 cells/mL
Culture type: Suspension

Culture vessels: TPP tubes, shaker flasks, or 3 L bioreactor
Recommended parameters for different culture vessels:

TPP Tubes	
Items	**Recommended Parameters**
50 mL TPP tubes	Culture Volume: 10–30 mL
Shaking Speed	200 rpm @50mm orbital throw shaker
Culture Temperature	37°C
CO_2 Concentration	5%
Relative Humidity	60%–80% RH
125 mL Shaker Flask	Culture Volume: 10–40 mL
250 mL Shaker Flask	Culture Volume: 40–80 mL
500 mL Shaker Flask	Culture Volume: 100–200 mL
1000 mL Shaker Flask	Culture Volume: 200–300 mL
Shaking Speed	125–135 rpm @25 mm orbital throw shaker
Culture Temperature	37°C
CO_2 Concentration	5%
Relative Humidity	60%–80% RH

3 L Bioreactor	
Items	**Recommended Parameters**
Shaking Speed	200 rpm
Culture Temperature	37°C
CO_2 Sparger	0.01 VVM
Air Sparger	0.01 VVM
O_2 Sparger	The initial setting is 0.02 VVM. It should be upregulated according to the increase of viable cell density.
pH	7.2 ± 0.2
DO	30%–50%
Note: An additional 1.0 g/L Pluronic F-68 is recommended to protect cells from shear force during bioreactor culture.	

Recover Cells

The cryopreserved volume is 1.0 mL/ tube, the cryopreserved density is 3.0×10^7 cells/mL, and the cells are recovered into 50 mL TPP tubes for suspension culture as an example.

1. Remove the cryopreserved tube from the liquid nitrogen, rapid thaw (<1 minute) the frozen cells in 37°C water bath, avoid immersing the lid of cryopreserved tube into the water.
2. Transfer all cell fluid into a sterile centrifuge tube with 30 mL prewarmed CD PK15 259 medium, centrifuge the

cells at 150–300 × *g* (about 800–1150 rpm) for 5 minutes, aspirate and discard the supernatant, then resuspend cells with 30 mL fresh prewarmed CD PK15 259 medium and transfer them into a new sterile TPP tube. Determine viable cell density (x 10^6 cells/mL) and viability (%) using cell counter equipment.

3. Culture the cells referring to the “Culture Conditions”. 24 ± 4 hours after recover, sample cell fluid to determine viable cell density (x 10^6 cells/mL) and viability (%) using cell counter equipment. If the cell viability <80%, add 10 mL fresh prewarmed CD PK15 259 medium to continues culture.
4. Repeat the operation of step 3 for 48 ± 4 hours recover. Taking sample of cell fluid to determine viable cell density (x 10^6 cells/mL) and viability (%) using cell counter equipment.
5. Passage the cells every 72 ± 4 hour, and passage method should refer to the steps of “Cell Passage and Scale-up”. The other experiments should be carried out after 3–5 continuous passages after cells growth stable.

Cell Passage and scale-up

The cells which are cryopreserved in CD PK15 259 medium and after successful recovery, or have been adapted to CD PK15 259 medium can be conducted continuous passage directly, and scale-up the cells according to the needs of subsequent experiments. The steps of cell passage and scale-up are as follows:

1. Taking sample of cells fluid to determine viable cell density (x 10^6 cells/mL) and viability (%) using cell counter equipment. Calculate required volume of CD PK15 259 medium and the cell fluid according to the seeding density of 0.6–0.8 × 10^6 cells/mL.
2. Taking required volume of cells fluid according to calculate result, resuspend cells with prewarmed CD PK15 259 medium.
3. Culture cells referring to the “Culture Conditions” It is recommended that viable cell density (× 10^6 cells/mL) and viability (%) should be determined every day. If the cell viability is <90%, centrifuge the cells at 150–300 × *g* (about 800–1150 rpm) for 5 minutes, aspirate and discard the supernatant, then resuspend cells with prewarmed fresh CD PK15 259 medium equal to the working volume. Otherwise, put it back into the incubator and continue to culture.
4. Before each passage, if the viable cell density <2.0 × 10^6 cells/mL or the viability <90%. It is need to conduct centrifuge passage, take out the required volume of cells fluid according to the calculation, centrifuge at 150–300 × *g* (about 800–1150 rpm) for 5 minutes, discard the supernatant and add culture medium, adjust the cell density to 0.6–0.8 × 10^6 cells/mL and continue to culture. Otherwise, follow the step of "Cell Passage and scale-up" to passage.
5. Passage the cells every 72 ± 4 hour. It is recommended other experiments should be carried out after 3–5 continuous passages after cells growth stable.

Adapt Cells to CD PK15 259 Medium

Adaptation is recommended if PK-15 cells are transferred from a different medium or culture type into CD PK15 259 medium, there are direct adaptation and sequential adaptation.

Direct Adaptation

PK-15 cells in good condition are conducive to direct adaptation, and it is recommended to use cells in logarithmic growth stage and transfer cells grown in other media into CD PK15 259 medium as following method:

1. Taking sample of cells fluid to determine viable cell density (× 10^6 cells/mL) and viability (%) using cell counter equipment. Calculate required volume cell fluid according to the seeding density of 0.6–0.8 × 10^6 cells/mL.
2. Taking sample of cells fluids according to the calculation results, centrifuge the cells at 150–300 × *g* (about 800–1150 rpm) for 5 minutes, aspirate and discard the supernatant, then resuspend cells with the fresh prewarmed CD PK15 259 medium (The volume equal to required working volume that need to passage), and seed the cells into a new sterile vessel.
3. Culture the cells referring to the “Culture Conditions”. Determine viable cell density (× 10^6 cells/mL) and viability (%) every day after cells first transfer to CD PK15 259 medium.

4. Passage the cells every 72 ± 4 hour, and the method should refer to the steps of “Cell Passage and Scale-up”. The other experiments should be carried out after 3–5 continuous passages until cells growth stable.
5. It is recommended that cells in the original medium were retained until successful adaptation in CD PK15 259 medium. Sequential adaptation should be used if the cell couldn’t growth well during direct adaptation.

Sequential Adaptation

Transfer PK-15 cells grown in other media to CD PK15 259 medium carried out sequential adaptation as follows:

1. The process of sequential adaptation needs to be carried out step by step in different proportions of the original medium mixed with CD PK15 259 medium. The mix proportions of the original medium (including the original medium and serum) and CD PK15 259 medium ratio are 90 : 10, 75 : 25, 50 : 50, 10 : 90, 0 : 100, at least 2- 3 passages should be carried for each mixed medium.
2. On the day of passage, determine viable cell density (× 10^6 cells/mL) and viability (%), calculate the volume of culture medium and cell fluid required for cell passage according to the inoculation density of 0.6–0.8 × 10^6 cells/mL and seed the cells into a new sterile vessel.
3. The passage of sequential adaptation process is carried out according to the steps of “Cell Passage and Scale-up”. The cells culture is referring to the “Culture Conditions”.
4. When PK-15 cells were completely transferred to 100% CD PK15 259 medium and after successful adaptive, carry on the passage and scale-up referring to “Cell Passage and Scale-up”. Add the other experiments should be carried out after 3–5 continuous passages until cells growth stable.

Cryopreserve Cells

Cryopreservation is recommended for cells in mid-log phase of growth with viability >90%.

1. Put the freezing container with fresh isopropanol, the cryopreserved tubes and other required materials into 2–8°C for pre-cooling for 24 hours or −20°C for pre-cooling for 2–4 hours in advance.
2. Determine viable cell density (× 10^6 cells/mL) and viability (%) using cell counter equipment, The cryopreserved volume is 1.0 mL/ tube, the cryopreserved density is 2.0–3.0 × 10^7 cells/mL cells/mL to calculate and prepare related liquids according to the following table:

Items	Formula
Required volume of cryopreservation solution (mL)	20% DMSO (mL) + 80% CD PK15 259 Medium (mL)
Required volume of cell resuspension fluid (mL)	Adaptive culture medium (mL)
Required total number of cells (× 10^7 cells)	Cryopreserved density (× 10^7 cells/mL) × Required number of cryopreserved tubes (tubes) × Cryopreserved volume/ tubes (mL)
Required volume of cell fluid (mL)	Required total number of cells (× 10^7 cells) ÷ The determined viable cell density (× 10^6 cells/mL)
Required volume of cryopreserved cell suspension (mL)	Cryopreserved volume/ tubes (mL) × Required number of cryopreserved tubes (tubes) Required volume of cell suspension (mL) + required volume of cryopreservation solution (mL)

3. Sample the cell fluid according to the calculation results, centrifuge the cells at 150–300 × *g* (about 800–1150 rpm) for 5 minutes, transfer the supernatant to a suitable sterile container for storage (This is adaptive culture medium), then gently pat the bottom of the centrifuge tube to make the cell precipitation evenly dispersed. Add the required volume of adaptive culture medium for resuspension according to the calculation results and prepare the cell suspension.
4. Add the pre-cooled cryopreservation solution drop by drop into the cell suspension to prepare the cryopreserved cell suspension, and gently shake while adding to ensure the uniformity of cells. This step needs to be carried out in the ice bath.
5. Place the pre-cooled cryopreservation tubes on the ice, and the cryopreserved cell suspension are divided into 1 mL of volume for each tube. During the loading period, the cryopreservation cell suspensions were mixed with light

shaking repeatedly.

6. After the separation, transfer the cryopreserved tubes to the pre-cooled freezing container, store it in the −80°C refrigerator for at least 24 hours, and transfer it to the liquid nitrogen tank for long-term storage.

Adapt Adherent Cell to Suspension Cell

1. Digest adherent PK-15 cells and then seed the cells into CD PK15 259 completed media at a seeding density of 1.5–2.0 × 10^6 cells/mL and culture referring to the “Culture Condition”.
2. After 72 ± 4 hours, the cells can be passaged directly when viable cell density more than 4.0 × 10^6 cells/mL and viability more than 90%. Otherwise, centrifugal passage is used.
3. Centrifuge the cells at 1000 rpm (about 233 × *g*) for 5 minutes, aspirate and discard the supernatant. Adding the required volume of CD PK15 259 completed media and culture referring to the “Culture Condition”.
4. The interval between cell passages is 72 ± 4 hours, once the cells showed a tendency to proliferate, the seeding density was changed from 1.5–2.0 × 106 cells/mL to 1.0 × 106 cells/mL.
5. After successful adaptation, PK-15 suspension cells can reach viable cell density of about 8.0 × 10^6 cells/mL in 72 hours.